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Correlation Between P-Selectin Level and Platelet Aggregation in Cerebral Venous Sinus Thrombosis Patients

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Abstract

One of the causes of cerebral venous sinus thrombosis (CVST) is platelet hyperactivity. Adhesion and secretion are the beginning of platelet activation, which is indicated by a change in the Platelet-selectin (P-selectin) level. The end result of platelet activation is platelet aggregation. However, it is unknown whether the beginning of platelet activation ends with platelet aggregation. This study aimed to discover the correlation between P-selectin level and platelet aggregation in CVST. This study used a cross-sectional escriptive observational correlative approach. Subjects were the CVST outpatients visiting the Department of Neurology Dr. Hasan Sadikin General Hospital, Bandung, Indonesia, from July to September 2021. A total of 49 subjects met the inclusion and exclusion criteria. This study used citrate plasma samples for platelet aggregation and serum for P-selectin assessment. Platelet aggregation were assessed using the light transmission platelet aggregation method while P-selectin was assessed using Enzyme-linked immunosorbent assay (ELISA). Platelet aggregation median was 10.6% (range 0.2–82.4%), which reflected normoaggregation. Platelet hyperaggregation were seen in 9 samples (8.4%). Median of P-selectin was 2.4 ng/mL (range 0.1–10.1 ng/mL) which were normal. High P-selectin level was observed in 16 (32.7%) with 4/16 (25%) experiencing platelet hyperaggregation. Statistical analysis showed a weak negative correlation between P-selectin and platelet aggregation (r=-0.012; p=0.467). In conclusion, no correlation is seen between P-selectin and platelet aggregation, which may be due to the fact that platelets are influenced by many factors that are not examined in this study.

Keywords: Adenosine diphosphate (ADP), cerebral venous sinus thrombosis (CVST), platelet aggregation, platelet-selectin (P-selectin)

Introduction

Cerebral venous sinus thrombosis occurs when a thrombus (blood clot) forms in the brain's venous. Thrombus in CVST forms due to inflammation caused by specific risk factors such as coagulation disorders, autoimmune diseases, acute infections, hormonal disorders, and trauma due to head injuries.[1,2]

Cerebral venous sinus thrombosis cases worldwide are estimated to occur annually in 3-4 people per 1 million population. The patients are more common among young adults rather than elderlies. The disease is more common in females than males (2.9:1).[3]

Impaired blood flow due to venous system blockage will cause increased pressure on brain tissues. Increased pressure on brain tissues will cause brain edema around the venous system blockage area. Capillaries and arterioles will rupture and result in cerebral parenchymal hemorrhage. Bleeding may spread to the nearest subarachnoid space if the pressure increases, causing reduced consciousness or even death.[1,2]

A thrombus or blood clot may form in the vein, artery, heart, or microcirculation. The thrombus may be caused by a deficiency of antithrombin III, proteins C and S, the presence of V Leiden factor, which leads to resistance to protein C activation, direct injury to cerebral sinuses, meningitis, and other prothrombotic disorders such as platelet aggregation. Platelet aggregation may cause approximately 22–25% CVST.[4-6]

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Thrombosis in CVST is generally caused by impaired platelet function and coagulation factors. Abnormalities in platelet activation in CVST cases may be impaired adhesion function, impaired release reactions or secretion, and impaired aggregation function. Due to inflammation, dysfunctional and impaired endothelial structure will cause platelet adhesion, which causes platelets to stick together with collagen in endothelial cells. Then, a reaction will occur to release platelet granules such as ADP, adenosine triphosphate (ATP), norepinephrine, and others to strengthen the aggregation of platelets stuck with collagen. The final process in this thrombosis process is platelet aggregation.[4-6]

Platelet-selectin is a type 1 transmembrane protein on platelet granules and megakaryocyte granules. Activated platelets will experience granular fusion with the endothelial plasma membrane, causing exposure of P-selectin on the surface of the activated platelets. Platelet-selectin also plays a role in mediating interactions between leukocytes and ligand that helps the leukocytes and platelets adhesion process, namely P-selectin glycoprotein ligand-1 (PSGL-1). Platelet-selectin glycoprotein ligand-1 brings leukocyte and platelet cells to attach to the damaged endothelium, which ultimately causes platelet aggregation to occur to plug the wound caused by inflammation. Platelet-selectin causes adhesion and secretion of granules, which in turn causes platelet aggregation.[7,8]

Coagulation factors also contribute to activating platelets. The amount of platelet membrane glycoproteins and the expression of plasma adhesive molecules significantly increased, and many procoagulant platelet factors are released into the blood. Thrombosis can significantly increase the release of P-selectin and other signals of platelet activity. Platelet selection is progressively expressed on the surface of platelets, binding them through the lectin domain to the binding site on adjacent platelets, stabilizing the interaction between bridged platelets and thus enabling the formation of stable, large platelet aggregation. Therefore, the level of platelet aggregation in thrombosis patients increased.[6-8]

It is unknown whether the beginning of platelet activation ends with platelet aggregation. The study aimed to analyze the correlation between the P-selectin level test and platelet aggregation in CVST patients.

Methods

Subjects in this research were CVST outpatients at the Dr. Hasan Sadikin General Hospital Bandung from July to September 2021. Subject inclusion criteria were ≥18 years old, while a history of <200,000/uL platelets became the criteria for exclusion. The research material was 10 ml of patients' plasma for platelet aggregation examination. This blood specimen must be processed within 2 hours, then centrifugated to obtain Platelet Rich Plasma (PRP), and then ADP was added to the PRP. Other materials included 2 ml of patients' serum for P-selectin level examination. Platelet aggregation used ADP 1 μM agonist with light transmission platelet aggregation method. Examination of P-selectin used the enzyme-linked immunosorbent assay (ELISA) method.

This research was a correlative, descriptive observational research with a cross-sectional design. The research data that has been collected was analyzed using Statistical Package for the Social Science (SPSS). Spearman's Rank Correlation test was used for statistical analysis to analyze the correlation between P-selectin level and the result of platelet aggregation examination in CVST patient.

Ethical approval has been received from the Dr. Hasan Sadikin General Hospital Health Research Ethics Committee No. LB.02.02/X.6.5/163/2021. The study has received permission from the Director of Human Resources for Education and Research at Dr Hasan Sadikin General Hospital Bandung No. LB.02.01/X.2.2.1/13394/2021..

Results

A total of 50 research subjects met the inclusion criteria and signed informed consent during the research. One subject was excluded due to a history of 145,000/uL (<200,000/uL) platelet count one month prior. The total number of subjects in this research was 49 patients.

The study used Shapiro-Wilk's normality test on platelet count, aggregation with ADP 1 μM stimulation, and P-selectin data. The normality test result showed abnormal data distribution ($p<0.05$). Thus, non-parametric statistics was used as an analytical approach by presenting data in median and range.

Data on research subject characteristics are presented in Table 1. Data from laboratory examination results of research subjects are presented in Table 2.

Table 1 Research Subject Characteristics Data

Characteristics	Median (Range)	n(%)
Age (years)	48 (22–68)	
Sex		
Male		11 (22.4)
Female		38 (77.6)
Symptom		
Headache		44 (89.8)
Weak on 1 extremity side		8 (16.3)
Seizure		1 (2)
Fainting		2 (4.1)
Others		12 (24.5)
Symptom duration (years)	3 (1–30)	
Risk Factor		
Head injury		5 (10.2)
Autoimmune		8 (16.3)
Hormonal		6 (12.2)
Infection		3 (6.1)
Malignancy		3 (6.1)
Other diseases		10 (20.4)
No risk factor		17 (34.7)
D-Dimer Before Therapy (mg/L)	0.3 (0.2–11.4)	
Abnormal		13 (30.2)
Normal		30 (69.8)
Radiology		
No examination history		3 (6.1)
MRV		23 (46.9)
DSA		9 (18.4)
CT Scan		14 (28.6)
Therapy		
Anticoagulant		45 (91.8)
Antiplatelet		4 (8.2)
Others		30 (61.2)
Duration of therapy (years)	2 (1–8)	

Notes: n: frequency, %: percentage, MRV: magnetic resonance venography; DSA: digital subtraction angiography, CT Scan: computed tomography scan

Table 2 Research Subject Laboratory Characteristic Data

Characteristics	Median (Range)
Platelet (x1000/uL)	352 (204–606)
Aggregation with ADP 1 µM stimulation	10.6 (0.2–82.4)

Notes: n=frequency, %=percentage

Table 3 Correlation between P-selectin Level and ADP 1 µM Platelet Aggregation Test

Research Variable	P-selectin (ng/mL)		
	n	Coefficient r	p-value
ADP 1 µM (%)	49	-0.012	0.467

Notes: Analysis uses Spearman's Rank correlation, *is significant, p<0.05

Normality test result using Shapiro-Wilk's test showed that data was not normally distributed, thus non-parametric Spearman's Rank correlation analysis was used. Table 3 showed the correlation between P-selectin and platelet aggregation based on ADP 1 µM level.

The correlation analysis result showed that there was a very weak negative correlation, and can be neglected, between P-selectin with ADP 1 µM (p>0.05). Figure 1 showed the Bland Altman scatterplot correlation between P-selectin and platelet aggregation test with AD.

The scatterplot above showed no correlation between P-selectin and platelet aggregation test with ADP 1 µM.

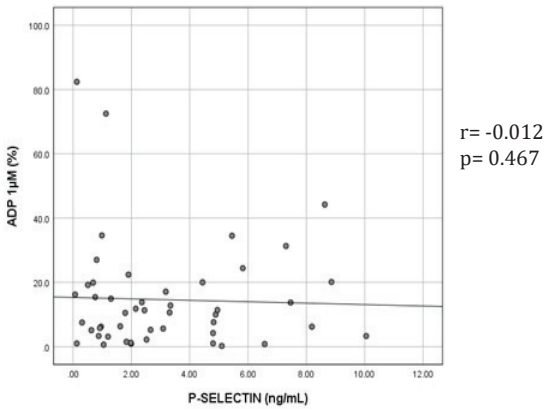

Figure 1 Correlation Scatterplot between P-selectin and Platelet Aggregation ADP 1 µM Test

Discussion

Based on the literature study, this research was the first in Indonesia to analyze the correlation between P-selectin level and the result of platelet aggregation examination in CSVT patients. The median age of research subjects was 48 years old, with a range of 22–68 years old in accordance with research by Ibrahim et al.,[4] who obtained 50% of subjects aged ≥40 years old. Research by Sidhom et al.[9] showed a mean age of 41 years old.This age range is categorized as reproductive age, where estrogen levels in subjects can add to the risk factors for CSVT. Estrogen will decrease by 2% each year entering menopause.

Female comprised the majority of research subjects (77.6%), following the research subjects of Krajickova et al.,[10] where 72.5% was female.Estrogen plays an important role in the occurrence of CVST. Estrogen as a risk factor in females increases coagulation factors and decreases the level of coagulants such as antithrombin. The hormone activates endothelium which interferes with endothelial permeability and causes endothelial damage, resulting in CVST due to thrombus formation. Estrogen also inhibits CYP1A2 and 2C19 and thus it is necessary to increase warfarin dose in CVST patients.[4]

The most frequent symptom in this study was headache (89.9%), with a median onset of 3 years and a range of 1–30 years. The symptom was progressive and persistent and did not improve with some therapy. Headache is caused by venous system blockage, resulting in impaired blood drainage and increased pressure in brain tissues due to a blocked venous system in the dural sinuses. Increased pressure will cause brain edema in the area involved. If the pressure is highly increased, high intracranial pressure will occur and results in the symptom of headache.[10,11]

The majority risk factor was autoimmune diseases (16.3%). This study followed the research of Zhang et al.,[11] who stated that autoimmune diseases caused by an immune response to antigens are an important cause of CVST. Autoimmunity causes inflammatory activation of vascular endothelium and adhesion of leukocyte and platelet components on the endothelium. Autoimmunity leads to hypercoagulability and easily damaged endothelium, which results in thrombosis.

The range of treatment (1–8 years) made variations in P-selectin results. The administration of drugs mainly consisted of anticoagulants (91.8%) and antiplatelets (8.2%). Aside from anticoagulants or antiplatelets, this research's subjects comprise several other drugs, such as steroids for autoimmune diseases, dyslipidemia, hypertension drugs, etc. All subjects have been given therapy; thus, their treatment history was not a confounding factor but became findings in this research.

Anticoagulants are necessary for CVST to prevent thrombus growth, facilitate recanalization, and prevent the risk of DVT. The subjects consumed Warfarin, an oral anticoagulant that is commonly used. The drug affects vitamin K synthesis, which contributes to blood clotting, resulting in the depletion of factors II, VII, IX, and X. Warfarin acts in the liver by inhibiting vitamin K carboxylation from its precursor protein. Due to the half-time of each blood clotting factor, depletion of factor VII will prolong the prothrombin time. As mentioned earlier, the anti-thrombotic effect only reaches its peak after the depletion of the four factors. The anticoagulant effect of warfarin will require several days.[12]

Antiplatelet drugs can affect the result of platelet aggregation examination, but in this research the subjects still consumed the drug due to the difficulty in obtaining research subjects. Aspirin works by irreversibly acetylating Ser529 in cyclooxygenase (COX-1), inhibiting the formation of thromboxane A2 (TXA2). Thromboxane A2 is a potent platelet aggregation that may strengthen platelet aggregation. Platelet aggregation inhibition due to aspirin consumption is irreversible because it occurs according to the age of platelets. Other antiplatelets, such as Clopidogrel, act on the adenosine receptor as the antagonist. Clopidogrel irreversibly inhibits the P2Y12 receptor, one of the adenosine receptors on platelets. Clopidogrel inhibits ADP-induced platelet aggregation.[13]

Endothelial dysfunction due to inflammation in CVST will cause the occurrence of platelet activation as well as an impaired coagulation system. Activation of platelets can be demonstrated by an increased level of P-selectin as well as platelet aggregation. Platelet aggregation will cause fibrin clots. Fibrin clot degradation will occur to maintain fibrinolytic system balance. Among fragments produced during fibrin clot degradation is D-dimer. Clinicians use D-dimer as a diagnostic parameter in CVST management.[14]

The median D-dimer history was 0.3 mg/L with a range of 0,2–11,4 mg/L, and 30,2% of subjects had increased D-dimer. A well-

designed prospective study on 343 patients showed that D-dimer levels decreased over time since symptom onset. In contrast, patients with sub-acute or chronic symptoms produced normal D-dimer results. Anatomic expansion of thrombosis sinus location in patients with extensive obstruction may also lead to a false low D-dimer result.[14]

Hyperaggregation due to ADP 1 µM stimulation was present in 18.4% of subjects, which is to the etiology of CVST that 22–25% of thrombosis is caused by platelet hyperaggregation in cerebral vessels.4-6 Platelet hyperactivity in thrombosis patients causes hyperaggregation to quickly occur when administering a low dose of an agonist such as ADP 1 µM.[15] Therefore, this research measured platelet aggregation with ADP 1 µM stimulation.

P-selectin level, according to the insert kit, is divided into 3 categories in which 0.47–0.53 ng/mL make up the low category, 0.97–1.03 ng/mL as the normal category, and 3.50–3.90 ng/mL as high category.[16] This research showed high P-selectin levels in 16 subjects, but most subjects showed normal P-selectin levels in 22 subjects and low levels in 11 subjects.

Platelet aggregation occurs due to the hyperactivity of platelets caused by active platelets, which is demonstrated by the increased level of P-selectin.[17] The lack of correlation between P-selectin level and platelet aggregation results in research subjects may be caused by many factors.

Many factors influence platelet aggregation. After platelets' initial contact with the subendothelial extracellular matrix, which is mediated by Von Willebrand Factor (VWF) and Glycoprotein Ib (GPIb), platelet adhesion occurs mediated by integrin and multiple cellular activations such as P-selectin. Then, platelet activation occurs by collagen, secretion of mediators such as ADP, thromboxane A2, or thrombin, and platelet shape changes. Mediators are still necessary to recruit platelets into the platelet plug to result in platelet aggregation in CVST patients.[18]

Not all platelets will be activated with the presence of a stimulus. Research by Wild et al. stated that according to standards, there are only 36% of activated platelets in platelet concentrate storage.[19] Based on this, there are variations in platelet activity, namely increased P-selectin level and platelet hyperaggregation due to activated platelets, as well as P-selectin level that does not increase and platelet normoaggregation or hypoaggregation because platelets are not activated. This causes variety in P-selectin level and platelet aggregation.[15]

ADP 1 µM agonist is reversible; thus, there is a possibility that a platelet plug will not form. In a low ADP level (1 µM), the light inductor will slightly increase and decrease due to the reversible aggregation. Platelets quickly experience deaggregation.[20] GPIIb/IIIa complex activation results in fibrinogen binding and platelet aggregation that may occur with a lower agonist level than required to produce degranulation. Stimulated platelets do not always lead to a fully irreversible aggregation. Meanwhile, P-selectin expression is a reversible process. Thus, P-selectin can be released from platelets during this phase and increases in subjects.[7,8]

Platelet activity includes increased P-selectin level after 1 hour and a maximum of 40 hours; according to research by Stokol et al.[21] Aggregated platelets can only survive up to 10 days throughout the platelet's life cycle.[15,20] Research by Lukasik et al.[18] showed that P-selectin may significantly decrease after a stroke. This research showed that P-selectin increases in acute and subacute stroke but decreases to normal in the 90th day (3 months). Research by Van Golen et al.[20] showed that platelet activity did not increase after liver ischemia, and thus, there was no increase in P-selectin level.

There are multifactorial risk factors underlying CVST such as autoimmune, hormonal therapy, head injury, infection, malignancy, and other diseases. These risk factors can also affect the result of P-selectin level examination using the ELISA method.[22]

The research concludes that no correlation exists between P-selectin level and platelet aggregation due to the many factors influencing the different results between the two variables. Further research is recommended to determine the relationship between P-selectin level and platelet aggregation test in CVST patients in subjects who have not received anticoagulant and antiplatelet therapies or with P-selectin level examination using other methods such as flow cytometry.

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